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Cell-cell fusion in the nervous system: Alternative mechanisms of development, injury, and repair

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ABSTRACT

Over a century ago, the seminal work of Ramón y Cajal revealed that the nervous system is made of individual units, the neurons, which are related to each other by contiguity rather than continuity. This view overturned the idea that the nervous system was a reticulum of fibers, a *rete diffusa nervosa*, as proposed and defined by Camillo Golgi. Although the neuron theory has been widely confirmed in every model system studied and constitutes the basis of modern neuroscience, evidence accumulated over the years suggests that neurons, similar to other types of cells, have the potential to fuse their membranes and undergo cell-cell fusion under certain conditions. This concept adds a substantial layer to our view of the nervous system and how it functions. Here, we bring together past and more recent discoveries on multiple aspects of neuronal fusion, discussing how this cellular event is generated, and what consequences it has for our understanding of nervous system development, disease, injury, and repair.

Contents

Abbreviations: AFF-1, anchor cell fusion failure-1; AMsh, amphid sheath; AMso, socket cells; DRG, dorsal root ganglia; EFF-1, epithelial fusion failure-1; gB, glycoprotein B; HSV1, herpes simplex virus type 1; PrV, pseudorabies virus; PS, phosphatidylserine; TNTs, tunnelling nanotubes; VZV, varicella-zoster virus.

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1. Introduction

The neuron theory proposed by Ramón y Cajal, according to which neurons exist as individual cells, has been the foundation of modern neuroscience and has paved the way for our current understanding of how the nervous system develops and functions. Neurons are highly polarized cells that extend two functionally and morphologically different compartments from the soma: dendrites and an axon. Dendrites are specialized to receive and process electrochemical inputs, whereas the axon transmits the electrochemical messages to other neurons or a target tissue via chemical or electrical synapses. Although a number of other tissues normally develop through cell-cell fusion, thereby generating multinucleate syncytia (placenta, muscle, osteoclasts, macrophages), this cellular mechanism was thought to be absent from the nervous system. However, several reports suggest that neurons, similar to other cells, have the capacity to fuse their plasma membranes, and that these events occur under both physiological and pathological conditions. This raises a number of intriguing questions. Is it possible that the true extent of neuronal fusion across species has been overlooked due to the complexity of the nervous system? What consequences do these cellular events have for the identity and function of the neurons involved, and for the circuits they form? Could neuronal fusion be part of the etiology underlying certain neurological diseases? What is the molecular machinery that governs neuronal fusion? This review aims to present the current evidence in the field, and to highlight the potential impact of recent advances on our understanding of the nervous system.

2. Neuronal self-fusion

One of the simplest examples of the capacity of neurons to fuse their membranes is observed during neuronal self-fusion. This term defines the ability of a neuron to fuse sections of its own neuronal processes (dendrites or axon), in order to remodel them during development or repair them following injury (Fig. 1 and Table 1).

2.1. Dendrite fusion to remodel a developing dendritic arbor

The PVDs (left and right) are a bilateral pair of mechansensory neurons in *Caenorhabditis elegans*, each of which extends anteriorly and posteriorly directed dendrites, and a ventrally directed axon. Each dendrite extends several multibranched units (named menorahs based on their shape) both ventrally and dorsally, covering almost the full body of the animal (Fig. 1A). This highly branched and stereotypical dendritic arbor has made PVD one of the best-characterized neurons in terms of dendrite development and repair [1–7]. Using this cell as a model system, Oren-Suissa and colleagues have described a new mechanism for dendritic arbor development involving membrane fusion [7]. They have elegantly shown that, during development, the PVD dendritic arbor is pruned and shaped through branch retraction and, most interestingly, through loop formation by neurite self-fusion. Both these processes were shown to be mediated by the nematode-specific fusogen Epithelial Fusion Failure-1 (EFF-1), a *bona fide* fusogen previously shown to mediate cell-cell fusion during development in other *C. elegans* tissues [8]. The authors proposed that assembly of EFF-1 complexes in *cis* causes membrane curvature and retraction, whereas interactions between EFF-1 molecules in *trans* across closely apposed membranes causes dendrite fusion. EFF-1 sculpts these neurons in a dose-dependent manner to maintain the angle of neurites at branching points and avoid overlapping branches. This process could be compared to the self-contact elimination process described decades ago for the development of neuronal growth cones *in vitro* [9]. A similar self-contact elimination by membrane self-fusion has recently been characterized in epithelial cells [10] and in the vascular endothelial cells of zebrafish embryos [11], raising the possibility that it might be a common mechanism to shape cellular processes (Fig. 1B). These findings show that some neurons express a functional fusogen, and that dendrites are capable of membrane fusion.

2.2. Axonal fusion to repair an injured axon

Another example of neuronal self-fusion is the process of axonal fusion observed during axonal regeneration. In this case, following transection of the axon, the proximal axonal fragment that is still attached to the cell body regrows toward and fuses with its own separated axonal fragment in an end-to-end or end-to-side configuration (Fig. 1C), re-establishing membrane and cytoplasmic continuity and therefore the original axonal tract. This process has been recognized for more than 50 years, and has been described in the motor neurons of crayfish [12], sensory neurons of the leech [13,14], giant axons of the earthworm [15], dissociated *Aplysia* sensory neurons *in vitro* [16] and, more recently, in the mechanosensory neurons of the nematode *C. elegans* [17,18]. In these studies, cytoplasmic continuity after rejoining of the two separated fragments was confirmed by electron microcopy [13,15,17,18], by injection of high molecular weight dyes (such as horseradish peroxidase) into the soma [14], or by expressing genetically encoded photoconvertible fluorophores such as Kaede [18], which were able to diffuse through the fusion site, from the soma to the distal axonal fragment. In some models, neuronal function has also been shown to recover fully at the electrophysiological [12,13,15,16] and behavioral levels [12,15].

Although the process of neuronal self-fusion during axonal regeneration has been well characterized at the morphological level, it was not until recent studies in *C. elegans* that the molecular mediators of this fusion process were identified [17,19]. In the *C. elegans* mechanosensory neurons, membrane fusion of the rejoining axonal fragments is mediated by the nematode fusogen EFF-1 [17,19]. In this process, EFF-1 is the final effector of a pathway involving changes in membrane lipid composition, which mediates the recognition of the separated distal fragment by the regrowing proximal fragment. In particular, these studies revealed that, following axonal transection, the lipid phosphatidylserine (PS) becomes exposed on the outer leaflet of the plasma membrane of the distal axonal fragment. Exposed PS itself, or PS bound by specific secreted ligands (such as the transthyretin TTR-52 or the lipid-binding protein NRF-5), is detected by transmembrane receptors present on the regrowing fragment (such as the PS receptor PSR-1, and possibly the TTR-52-binding receptor CED-1), thereby mediating recognition between the two separated axonal fragments prior to specific membrane fusion [19].

It is not known whether the role of these molecules in mediating axonal self-fusion during regeneration is conserved among species, but it is likely that similar molecular pathways are involved in other organisms, given that membrane fusion is an active process that requires specialized molecular players. PS exposure and recognition by cell surface receptors is a common mechanism for many cell-cell fusion events, and has been implicated in the fusion of myoblasts [20,21], syncytiotrophoblast cells in the placenta [22], and macrophages [23,24], as well as in the fusion that mediates the entry of some viruses into host cells [25,26]. Finally, it is likely that species-specific fusogens act as the last effectors in the mediation of membrane fusion. Taken together, these findings demonstrate that neurons of different classes and from different invertebrate species likely express functional fusogens and can fuse their membranes as a mechanism of repair.